data show that mixed green tea extract as well as its individual catechin components is effective in inhibiting breast cancer and endothelial cell proliferation in vitro and that green tea extract suppresses breast cancer xenograft size and decreases the tumor blood vessel density in vivo. In the present study, we further demonstrate that green tea extract or epigallocatechin-3-gallate at 40 μg/mL can decrease the levels of the angiogenic basic fibroblast growth factor in the cells. This phenomenon is observed in both human umbilical vein endothelial cells (HUVEC) and human breast cancer cells MDA-MB231. This effect is dose dependent. Furthermore, green tea extract and epigallocatechin-3-gallate decrease the transcription levels of basic and acidic fibroblast growth factors in HUVEC and MDA-MB231 cells. Our findings suggest that the inhibition of the angiogenic fibroblast growth factors could account for one of the mechanisms of green tea's actions. Because cancer is angiogenesis dependent, this may explain in part the antineoplastic effects associated with green tea consumption.

Changes in the Fecal Flora Composition of Human Volunteers in a Double-Blind Randomized Black Tea Feeding Study. V. Mai, H. Katki, B. Clevidence,* S. Hursting,† H. Harmsen and A. Schatzkin.** Division of Cancer Epidemiology and Genetics and †Division of Cancer Prevention, National Cancer Institute, Bethesda, MD; *Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD; and **University of Groningen, Groningen, The Netherlands.

The importance of the microbial intestinal flora in human cancer and other disease has long been postulated. The effects of some dietary substances, such as prebiotics, on the composition of the flora have been established. The effects of diet on health may be mediated in part by changes in the composition of the intestinal flora. Black tea has been suggested to affect cancer risk, although evidence for this association is equivocal. Polyphenols in black tea could alter the bacterial flora, leading to an increased excretion of carcinogenic fecal bile acids. We analyzed changes in the fecal flora composition and in the fecal bile acid profile in 15 subjects in a black tea feeding study. Fecal samples were collected on d 1, 13 and 20 of the two periods (crossover design) for a total of six samples from each volunteer. We performed fluorescent in situ hybridizations (FISH) and temporal gradient gel electrophoresis (TGGE) to analyze the bacterial flora and determine changes in the bile acid profile enzymatically and by HPLC. Large inter- and intraindividual variation masked any small effects of diet or black tea on the flora composition. However, we did observe a decrease in the amounts of "other bacteria" detected only by the universal bacterial probe in the FISH analysis. TGGE analyses showed a distinct bacterial profile for each subject that was relatively stable over time. These results indicate that tea drinking affects some flora components, but more sensitive tools and larger studies are required to evaluate effects of diet on the intestinal flora.

Inhibition by Garlic Allyl Sulfides and Phenethylisothiocyanate of Methyl-*n*-Pentylnitrosamine Dependentylation by P450 and Microsomes and of Liver DNA Alkylation by Dimethylnitrosamine in Rats. L. Zhou, C. R. Morris, S. C. Chen and S. S. Mirvish. Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE.

Garlic and cruciferae are associated with reduced risks of some human cancers, and several garlic allyl sulfides and phenethylisothiocyanate (PEITC) are anticarcinogenic in animal tests. Here we studied effects of garlic oil components diallyl sulfide (DAS) and diallyl disulfide (DADS) and the cruciferae constituent PEITC on the metabolism of the rat esophageal carcinogen methyl-*n*-pentylnitrosamine (MPN) and the rat liver carcinogen dimethylnitrosamine (DMN). We reported that MPN dependentylation (its activation step) was inhibited by DAS (1). The method involved incubation of various [^{3}H]MPN and DAS concentrations with cytochrome P_{450} (CYP) or microsomes and determination of resultant [^{3}H]pentaldehyde by HPLC of its 2,4-dinitrophenylhydrazone. Inhibitory rate constants (K_i) were 0.2–2.0 μmol/L for rat and human CYP2E1 and rat CYP2B1. Using similar techniques, we now report that DAS inhibition of MPN dependentylation showed a K_i of 0.6 μmol/L for both rat CYP2A3 and rat esophageal microsomes; DADS showed a K_i of 7, 60–80, 1.4–2.0 and 0.5–1.7 μmol/L for rat CYP2E1, human CYP2E1, rat CYP2A3 and rat esophageal microsomes, respectively; and PEITC showed a K_i of 4, 3–6 and 0.1–0.6 μmol/L for rat CYP2E1, CYP2A3 and esophageal microsomes, respectively. DAS and DADS (but not PEITC) were preincubated with P_{450} and microsomes for 15 min before the addition of MPN because this increased inhibition for rat CYP2E1 by 40–70%. We studied allyl sulfide inhibition of DNA guanine methylation by DMN, following similar studies with garlic powder by Milner et al. (2). We introduced allyl sulfides in 5 mL corn oil/kg or corn oil controls by gavage into male 9- to 12-wk-old Sprague-Dawley rats, waited 2–18 h, injected DMN (20 mg in 5 mL saline/kg) intraperitoneally, killed the rats 3 h later, homogenized and lysed ~350 mg liver, purified its DNA on Qiagen Genomic tips and hydrolyzed DNA by standard methods. O^6-Methylguanine (O^6-MG) and guanine were determined by HPLC on a SCX cation exchange column with fluorescence detection. Corn oil controls showed ratios of O^6-MG to guanine of 0.066 ± 0.005% (A ± B = mean ± SEM). Inhibition of O^6-MG formation (expressed as ratios of O^6-MG to guanine, mainly 6 rats/group) was 39 ± 5% for 200 mg DAS/kg given 2 h before DMN; 88 ± 2% down to 15 ± 7% for 200 down to 6 mg DAS/kg given 18 h before DMN; and 53 ± 15%, 41 ± 3% and 36 ± 4% for 125, 75 and 50 mg DADS/kg, respectively, given 18 h before DMN. The effective inhibition for DAS given 18 h before DMN and metabolic studies by Yang et al. (3) suggest that DAS is metabolized to diallyl sulfone, which persists and irreversibly destroys liver CYP2E1 that activates DMN. In support of this view, diallyl sulfoxide (90 mg/kg) and diallyl sulfone (100 mg/kg), given 18 h before DMN injection, inhibited O^6-MG production in liver DNA from DMN by 72 ± 7% and 82 ± 10%, respectively, similar to the effect of DAS. Feeding 5% crushed garlic in a commercial diet for 3 d before DMN injection inhibited O^6-MG production in liver DNA by 45 ± 5%. These results help explain the inhibition of nitrosamine carcinogenesis by vegetable constituents. [Supported by American Institute for Cancer Research grant 00A080 and National Institutes of Health core grant P30-CA-36727.]

1. Morris, C. R., Yao, D., Chen, S. C. & Mirvish, S. S. (2000) Diallylsulfide inhibition of methyl-*n*-amylnitrosamine metabolism by P450s and fate of diallylsulfide and diallyldisulfide in rats. Proc. Am. Assoc. Cancer Res. 41: 661 (abs.).

2. Lin, X. Y., Liu, J. Z. & Milner, J. A. (1994) Dietary garlic suppresses DNA adducts caused by *N*-nitroso compounds. Carcinogenesis 15: 349–352.

3. Chen, L., Lee, M., Hong, J.-Y., Huang, W., Wang, E. & Yang, C. S. (1994) Relationship between cytochrome P450 2E1 and acetone catabolism in rats as studied with diallyl sulfide as an inhibitor. Biochem. Pharmacol. 48: 2199–2205.